NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 443

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF OXAZEPAM

(CAS NO. 604-75-1)

IN SWISS-WEBSTER AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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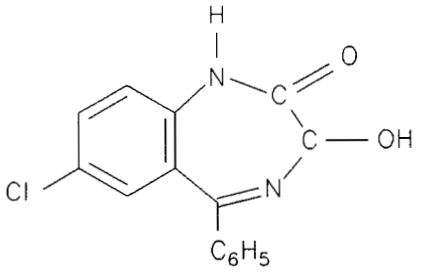
ABSTRACT



OXAZEPAM

CAS No. 604-75-1

Chemical Formula: $C_{15}H_{11}ClN_2O_2$ Molecular Weight: 286.74

Synonym: 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
Trade Names: Tazepam, Wy-3498, Serax

Oxazepam is one of a number of benzodiazepines used therapeutically as a sedative-hypnotic and anti-anxiety agent. Toxicology and carcinogenesis studies were performed by administering oxazepam (greater than 99% pure) in feed to male and female Swiss-Webster and B6C3F$_1$ mice for 14 weeks, 57 weeks (Swiss-Webster), or 2 years (B6C3F$_1$). Neurobehavioral assessments were performed during the studies. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells, and peripheral blood samples were analyzed for frequency of micronucleated normochromatic erythrocytes. Supplemental studies were performed to compare the metabolism and toxicokinetics of oxazepam in the two mouse strains, to evaluate the effect on liver cell replication rates, to perform clinical pathology assessments, and to examine the mutation spectrum and frequency of activated H-*ras* oncogenes in liver neoplasms from the 2-year study with B6C3F$_1$ mice.

14-Week Study in Swiss-Webster Mice

Groups of 10 male and 10 female Swiss-Webster mice received oxazepam in feed at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm for 14 weeks. One 625 ppm male and one 10,000 ppm female were killed moribund before the end of the study, and the condition of the female mouse was attributed to oxazepam exposure. Mean body weight gains of exposed groups were similar to those of the controls. Exposed mice displayed chemical-related sedation and lethargy during the first study week, but appeared normal thereafter. In the neurobehavioral studies, reductions in grip strength were evident in both male and female mice at week 2 and persisted in males through week 11. An antianxiety effect was detected in exposed mice in measures of motor activity, startle response, and reactions to thermal stimulus.

At necropsy, absolute and relative liver weights were increased in an exposure-related manner and were approximately two-fold greater in 10,000 ppm mice than in controls. Centrilobular hepatocellular hypertrophy was present only in exposed mice, and the severity increased with dose.

14-Week Study in B6C3F$_1$ Mice

Groups of 10 male and 10 female B6C3F$_1$ mice received oxazepam in feed at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm for 14 weeks. There were no deaths that were clearly related to oxazepam exposure. Mean body weight gains of exposed groups were similar to those of the controls.

Exposed mice displayed chemical-related sedation and lethargy during only the first study week. In neurobehavioral studies, reductions in grip strength were evident in males at week 2 but were no longer observed at week 12. An antianxiety effect was noted in exposed mice in measures of motor activity, startle response, and reactions to a thermal stimulus (females).

At necropsy, absolute and relative liver weights were increased in an exposure-related manner and were approximately two-fold greater in 10,000 ppm mice than in controls. Centrilobular hepatocellular hypertrophy was present only in exposed mice, and the severity increased with dose.

CHRONIC STUDIES

Groups of 60 male and 60 female Swiss-Webster and $B6C3F_1$ mice received oxazepam in feed at concentrations of 0, 2,500, or 5,000 ppm. Additional groups of 60 male and 60 female $B6C3F_1$ mice received 125 ppm in feed to allow for study of a group with projected serum concentrations of oxazepam similar to those achieved in humans taking a therapeutic dose. Ten male and 10 female $B6C3F_1$ mice per group were evaluated at 15 months. Average daily oxazepam consumption varied throughout the studies, and the overall daily average ranged from 10 to 29 mg/kg body weight for the 125 ppm groups, 234 to 512 mg/kg for the 2,500 ppm groups, and 444 to 1,085 mg/kg for the 5,000 ppm groups. Serum oxazepam concentrations determined at 57 weeks in Swiss-Webster mice and at the 15-month interim evaluation of $B6C3F_1$ mice were approximately 1 μg/mL in the 125 ppm groups, 4 to 7 μg/mL in the 2,500 ppm groups, and 7 to 10 μg/mL in the 5,000 ppm groups.

Neurobehavioral assessments during the chronic studies of each strain of mice were confounded by the poor survival and deteriorating condition of mice with hepatic neoplasia. However, within the limitations of the studies, there were no notable changes in the types of behaviors observed compared to those observed in the 14-week studies, nor was there an enhancement in the degree to which they were exhibited.

57-Week Study in Swiss-Webster Mice

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

At 57 weeks, survival of exposed mice was significantly lower than that of controls (males: 0 ppm, 45/60; 2,500 ppm, 19/60; 5,000 ppm, 10/60; females: 47/60, 28/59, 17/59), causing the study to be terminated. Mean body weights of exposed males were similar to controls until week 17; afterwards, mean body weights of exposed male groups were lower than those of controls. Final mean body weights of exposed males were 9% lower than that of the controls. The mean body weight of 2,500 ppm females was greater than that of the controls throughout the study. Females receiving 5,000 ppm had a mean body weight greater than that of the controls early in the study; after week 29, the mean body weight of this group was similar to that of the controls. Feed consumption by exposed males and females was slightly lower than that by the controls, and females in all groups, including controls, consumed slightly more feed than males throughout the study. Dietary levels of 2,500 and 5,000 ppm oxazepam resulted in average daily compound consumption levels of 270 and 570 mg/kg for males and 320 and 670 mg/kg for females. Hypoactivity and sedation were observed in exposed mice during the first week of the study. There were no other clinical findings associated with oxazepam exposure.

Pathology Findings

Systemic amyloidosis was the principal cause of death in mice dying before the study was terminated. The lower survival of mice receiving oxazepam was attributed to an increase in the extent and severity of amyloid deposits in many organs, including the heart and kidney. Atrial thrombosis and pulmonary lesions consistent with chronic heart failure occurred at higher incidences and with greater severity in exposed mice.

The incidence of hepatocellular adenomas (males: 1/60, 35/60, 50/60; females: 0/60, 22/59, 47/59) and carcinomas (males: 0/60, 5/60, 19/60; females: 1/60, 1/59, 11/59) were increased in exposed mice. The incidences of eosinophilic foci were also increased in exposed mice (males: 0/60, 22/60, 22/60; females: 0/60, 20/59, 14/59), and there was evidence of

increased centrilobular hepatocyte hypertrophy (males: 12/60, 46/60, 47/60; females: 3/60, 51/59, 53/59).

2-Year Study in B6C3F$_1$ Mice

Survival, Body Weights, Feed and Compound Consumption, and Clinical Findings

Survival of mice receiving 2,500 and 5,000 ppm was significantly lower than that of controls (males: 0 ppm, 45/50; 125 ppm, 44/50; 2,500 ppm, 15/50; 5,000 ppm, 0/50; females: 39/50, 41/50, 2/50, 0/50). Mean body weight gains of exposed male and female mice were similar to controls until about week 15 when weight gains for mice exposed to 2,500 or 5,000 ppm slowed in relation to controls, resulting in weight gains approximately 30% to 40% lower than those of the controls throughout the remainder of the study. Mean body weight gain of male mice exposed to 125 ppm was similar to that of the controls, while that of female mice receiving 125 ppm was 10% to 15% lower than that of the controls after about week 45. Feed consumption by exposed males and females was similar to that by controls. Dietary levels of 125, 2,500, and 5,000 ppm resulted in average daily oxazepam consumption levels of 12, 310, and 690 mg/kg body weight for males and 15, 350, and 780 mg/kg for females. In the 5,000 ppm groups, lethargy and sedation were observed in a few mice during the first week of study.

Pathology Findings

The early deaths of many of the B6C3F$_1$ mice exposed to oxazepam were attributed to a marked increase in the incidences of hepatoblastoma (males: 0/49, 2/50, 21/50, 13/50; females: 0/50, 1/50, 8/50, 8/50), hepatocellular adenoma (males: 17/49, 18/50, 34/50, 32/50; females: 25/50, 35/50, 35/50, 36/50), and hepatocellular carcinoma (males: 9/49, 5/50, 45/50, 50/50; females: 9/50, 5/50, 49/50, 44/50). Moderate hypertrophy of centrilobular hepatocytes occurred in mice receiving 2,500 and 5,000 ppm (males: 0/49, 2/50, 26/50, 43/50; females: 0/50, 2/50, 11/50, 29/50). An increase in the incidence of follicular cell hyperplasia of the thyroid gland occurred in all exposed groups of mice (males: 4/49, 22/50, 49/50, 47/50; females: 16/50, 34/50, 49/50, 44/50), and thyroid gland follicular cell adenoma was increased in exposed females (0/50, 4/50, 5/50, 6/50). Testicular atrophy occurred in the 2,500 and 5,000 ppm groups (1/50, 0/50, 25/50, 38/50), and the incidence of epididymal lymphocyte infiltration was increased in all exposed groups (2/50, 14/50, 33/50, 21/50).

The frequency of hepatocellular neoplasms with an activated H-*ras* oncogene in the B6C3F$_1$ mice and the mutation spectrum of the H-*ras* gene were determined. The mutation spectrum of the H-*ras* genes in the relatively few neoplasms from exposed mice that did have an activated H-*ras* did not differ from the spectrum of mutations observed in neoplasms from controls, but the proportion of neoplasms with an activated H-*ras* gene decreased with increasing oxazepam dose. While 11 of 19 (58%) neoplasms from control mice had an activated H-*ras* gene, only 1 of 40 neoplasms from mice receiving 2,500 or 5,000 ppm oxazepam exhibited a similar molecular lesion. Thirteen of 37 (35%) neoplasms from mice in the 125 ppm group had an activated H-*ras* oncogene, suggesting that, although the incidence of all liver neoplasms was not statistically increased compared to controls, there was an increase in a similar subset of neoplasms (lacking an activated H-*ras*) that occurred with increased incidence at higher doses.

SUPPLEMENTAL STUDIES

Because exposure to oxazepam caused increased incidences of liver neoplasms, supplemental short-term studies were performed. Oxazepam given in feed to male B6C3F$_1$ mice at 25, 125, 2,500, or 5,000 ppm for up to 13 weeks was found to cause a dose-related increase in nuclear labeling index in studies measuring the incorporation of bromodeoxyuridine into replicating liver cells. This increase was statistically significant at all but the 25 ppm exposure level and was limited to mice evaluated at 15 days. Cell replication rates in most groups evaluated at 30 days and after were similar to control rates. There was minimal evidence suggestive of hepatocyte necrosis either by light microscopy or in clinical chemistry measures. There was, however, evidence of cholestasis, likely due to physical obstruction of bile canaliculi by swollen hepatocytes.

The metabolic fate and toxicokinetics of oxazepam were evaluated in each strain of mice and were compared to published data from human studies.

Both mice and humans form glucuronides of oxazepam and form 3- and 4-hydroxy and methoxy derivatives of the phenyl group. Oxidative metabolism of the phenyl group appears to be more prevalent in mice than is reported for humans. Elimination half-lives of parent compound do not differ between Swiss-Webster and $B6C3F_1$ mice and are similar to values reported for humans.

GENETIC TOXICOLOGY

Oxazepam was not mutagenic in any of several strains of *Salmonella typhimurium*, nor did it induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* tests were performed with and without S9 metabolic activation. Results from an *in vivo* mouse peripheral blood micronucleus test performed on the $B6C3F_1$ mice used in the 14-week study were also negative.

CONCLUSIONS

Under the conditions of these feed studies, there was *clear evidence of carcinogenic activity** of oxazepam in male and female Swiss-Webster mice based on increased incidences of hepatocellular adenoma and carcinoma. There was *clear evidence of carcinogenic activity* of oxazepam in male and female $B6C3F_1$ mice based on increased incidences of hepatoblastoma and hepatocellular adenoma and carcinoma. Increased incidences of hyperplasia of thyroid gland follicular cells in male and female $B6C3F_1$ mice and of follicular cell adenomas in female $B6C3F_1$ mice were also related to oxazepam exposure.

Administration of oxazepam to Swiss-Webster mice resulted in centrilobular hepatocellular hypertrophy and increased incidences and severity of systemic amyloidosis. Administration of oxazepam to $B6C3F_1$ mice also resulted in centrilobular hepatocellular hypertrophy.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this report appear on page 12.

Summary of the Chronic Carcinogenesis and Genetic Toxicology Studies of Oxazepam

	Male Swiss-Webster Mice	Female Swiss-Webster Mice	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 2,500, or 5,000 ppm (approximately 270 or 570 mg/kg in feed)	0, 2,500, or 5,000 ppm (approximately 320 or 670 mg/kg in feed)	0, 125, 2,500, or 5,000 ppm (approximately 12, 310, or 690 mg/kg in feed)	0, 125, 2,500, or 5,000 ppm (approximately 15, 350, or 780 mg/kg in feed)
Body weights	Exposed groups lower than controls	2,500 ppm group higher than controls	2,500 and 5,000 ppm groups lower than controls	Exposed groups lower than controls
Survival rates[a]	45/60, 19/60, 10/60	47/60, 28/59, 17/59	45/50, 44/50, 15/50, 0/50	39/50, 41/50, 2/50, 0/50
Nonneoplastic effects	Multiple organs: increased incidence and severity of systemic amyloid deposition Liver: centrilobular hypertrophy (12/60, 46/60, 47/60)	Multiple organs: increased incidence and severity of systemic amyloid deposition Liver: centrilobular hypertrophy (3/60, 51/59, 53/59)	Liver: centrilobular hypertrophy (0/49, 2/50, 26/50, 43/50); Thyroid gland: follicular cell hyperplasia (4/49, 22/50, 49/50, 47/50)	Liver: centrilobular hypertrophy (0/50, 2/50, 11/50, 29/50); Thyroid gland: follicular cell hyperplasia (16/50, 34/50, 49/50, 44/50)
Neoplastic effects	Liver: hepatocellular adenoma (1/60, 35/60, 50/60); carcinoma (0/60, 5/60, 19/60)	Liver: hepatocellular adenoma (0/60, 22/59, 47/59); carcinoma (1/60, 1/59, 11/59)	Liver: hepatoblastoma (0/49, 2/50, 21/50, 13/50); hepatocellular adenoma (17/49, 18/50, 34/50, 32/50); carcinoma (9/49, 5/50, 45/50, 50/50)	Liver: hepatoblastoma (0/50, 1/50, 8/50, 8/50); hepatocellular adenoma (25/50, 35/50, 35/50, 36/50); carcinoma (9/50, 5/50, 49/50, 44/50); Thyroid gland: follicular cell adenoma (0/50, 4/50, 5/50, 6/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium gene mutation:	Negative in strains TA97, TA98, TA100, TA102, and TA1535 with and without S9			
Sister chromatid exchanges Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Micronucleated normochromatic erythrocytes in $B6C3F_1$ mice:	Negative at 14 weeks			

[a] Survival of Swiss-Webster mice based on a 57-week study; survival of $B6C3F_1$ mice based on 2-year study.

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such neoplasms to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign tumors have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign tumors of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific tumor;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on oxazepam on December 1, 1992, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On December 1, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of oxazepam received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of oxazepam by discussing the uses and rationale for study, describing the experimental design in Swiss-Webster and $B6C3F_1$ mice, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in both mouse strains. Dr. Bucher reported that due to the marked enhancement of liver neoplasia in both strains, a number of supplemental studies were performed at NIEHS including a study to evaluate rates of replicative DNA synthesis in the liver, metabolic fate and toxicokinetic studies, and analysis of the frequency of occurrence of an activated H-*ras* oncogene in hepatocellular neoplasms in $B6C3F_1$ mice. The proposed conclusions were *clear evidence of carcinogenic activity* of oxazepam in male and female Swiss-Webster mice and in male and female $B6C3F_1$ mice.

Dr. Ward, a principal reviewer, agreed with the proposed conclusions. He said it should be noted that in the $B6C3F_1$ mouse study, the two highest exposure levels exceeded maximum tolerated dose guidelines, but despite the severe depression in body weight gain, liver neoplasms were associated with early mortality and increased feed consumption. Dr. Bucher thought this was a reasonable point for further discussion by the Subcommittee. Dr. Ward said it was important to establish whether the thyroid follicular cell hyperplasia was goiter (diffuse) or focal (not diffuse) in $B6C3F_1$ mice. Dr. Bucher responded that at the two highest exposure levels, hyperplasia was a diffuse goiter type. Dr. Ward asked that the appendixes associated with the supplemental studies be discussed in the Results section.

Dr. Taylor, the second principal reviewer, agreed with the proposed conclusions. He complimented the inclusion of the mechanistic studies and also urged that the appendixes be discussed in the Results section. Dr. Taylor thought the detailed discussion of chlordiazepoxide genotoxicity was not necessary since little of this agent metabolized to oxazepam, while genotoxicity information might be useful on temazepam, which is metabolically converted largely to oxazepam. Dr. Bucher agreed to add genotoxicity information on temazepam if available. (Genotoxicity information was not available in the literature.)

Dr. Ryan, the third principal reviewer, agreed with the proposed conclusions. She said the different patterns of weight gain between male and female Swiss-Webster mice were of some concern, and wondered if these patterns could be explained through the varying incidences of toxicity and neoplasia. Dr. Bucher said there was not a clear-cut cause and effect relationship that would explain the differences. Dr. Ryan asked why no studies were conducted to assess reproductive toxicity since one of the rationales for the study was to examine the use of the drug by pregnant women. Dr. Bucher commented that adequate reproductive and developmental toxicology studies had been conducted as a part of the FDA drug approval process. Dr. Ryan noted that since the 125 ppm exposure level in $B6C3F_1$ mice was included in an attempt to produce a blood level in the therapeutic range for humans, interpretation of the findings for humans should be addressed in the Conclusions. Dr. Bucher said he would add a phrase that there were indications in the study that the amount of oxazepam was sufficient at that level to influence expression of the neoplastic process (page 62).

Dr. Davidson asked that some of the nonneoplastic lesions, notably heart lesions (amyloidosis) in Swiss-Webster mice and testicular lesions in $B6C3F_1$ mice, be summarized in the text along with the appropriate statistical analysis. Dr. Bucher explained that since the amyloidosis was a systemic effect, such a focus on the heart lesions could be misleading. With regard to the testicular lesions, he said it was likely that this was a treatment-related effect but could also be secondary to debilitation of the animal. There was no evidence from the 14-week study that the testis was a target organ. Dr. Davis thought there needed to be a clear presentation in the text of the toxicokinetic studies including area under the curve (AUC) information, noting that the extensive

amyloidosis in one strain of mice could affect chemical disposition depending on the organs involved. Dr. Bucher said AUC data were included, and noted that young Swiss-Webster mice were used for the toxicokinetic studies so amyloidosis would not have been present.

In comments from the public, Dr. Michael McClain, Hoffman-LaRoche, stated that the existence of the thyroid follicular cell hypertrophy along with hyperplasia of a diffuse type provided fairly clear evidence that the thyroid gland effects were probably secondary to hormone imbalance. Dr. Klaassen asked whether serum thyroid-stimulating hormone (TSH) levels had been measured.

Dr. Bucher replied that thyroid hormone status was not determined in the studies done to date, but there were plans to measure TSH and other thyroid hormones in further studies. In response to a question by Dr. Klaassen about measurement of P-450 isoforms, Dr. Julian Leakey, NCTR, reported that his laboratory was going to be doing studies in rats and mice treated with oxazepam, looking at induction of specific isoforms of P-450. Dr. Joseph Contrera, Center for Drug Evaluation and Research, FDA, praised the interaction between the FDA and the NTP in the design and conduct of oxazepam studies.

Dr. Ward moved that the Technical Report on oxazepam be accepted with the revisions discussed and with the conclusions as written for male and female Swiss-Webster mice and male and female $B6C3F_1$ mice, *clear evidence of carcinogenic activity.* Dr. Taylor seconded the motion, which was accepted by nine yes votes with one abstention (Dr. van Zwieten).